AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	10/31/12
Sponsor:	Sanosil International, LLC 91 Lukens Drive, Suite A New Castle, DE 19720
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Efficacy of a Disinfectant Applied to a Room Via a Fogger or Misting Device
ATS Labs Protocol Number:	SAN01101111.CUST
ATS Labs Project Number:	A14152

Modifications to Protocol:

A. On the day of testing, the Sponsor requested that the HVAC vents in the ceiling be covered and a humidifier/dehumidifier be used in the testing room to target 35-45% room humidity prior to running each cycle.

B. On each day of testing, the Sponsor instructed the technician to start the machine by holding the start button in for two seconds.

C. Per Sponsor request, the protocol is being amended to clarify the expiration dates for each lot of test substance. The expiration date of 3/21/12 for Lot 1109C is incorrectly listed in the protocol and it should be 3/21/2013. The expiration date for Lot 12I001 is 9/1/2013 and the expiration date for Lot 1206D is 6/30/2013.

Changes to the protocol are acceptable as noted.

Matthew Sathe
Study Director

10-31-12
Date

EXACT COPY
INITIALS MS DATE 12-11-12

(For Laboratory Use Only)
ATS Labs Project # A14152
KAS 10/18/12

ATS LABS

PROTOCOL

Efficacy of a Disinfectant Applied to a Room Via a Fogger or Misting Device

Test Organism:

Clostridium difficile - spore form (43598)

PROTOCOL NUMBER

SAN01101111.CUST

PREPARED FOR

Sanosil International, LLC
91 Lukens Drive, Suite A
New Castle, DE 19720

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

Matthew Sathe, B.S.
Senior Microbiologist

DATE

October 11, 2011
Revised Date: July 18, 2012
Final Revision: October 10, 2012

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INITIALS AS DATE 12-11-12

Efficacy of a Disinfectant Applied to a Room Via a Fogger or Misting Device

SPONSOR: Sanosil International, LLC
91 Lukens Drive, Suite A
New Castle, DE 19720

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE

The purpose of this assay is to determine the efficacy of a room bio-decontamination system using a disinfectant applied by a fogger or misting device in a sealed large volume enclosure.

TEST SUBSTANCE CHARACTERIZATION

Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY

Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is October 15, 2012. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of November 15, 2012. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs or any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the regulating agencies concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

Regulatory agencies require that specific sporicidal disinfection claims for a disinfectant product and bio-decontamination system intended for use on hard surfaces be supported by appropriate scientific data demonstrating the efficacy of the product and delivery system against the claimed test organism. The U.S. EPA typically requires the testing of 3 independent lots of test substance, one of which must be ≥60 days old at the time of test, to substantiate efficacy claims. Testing is accomplished in the laboratory by treating the test organism with the test substance under conditions which simulate as closely as possible the actual conditions under which the test substance is designed to be used. The experimental design in this protocol meets these requirements. However, this protocol has not been reviewed by regulatory agencies for registration compliance. Acceptance of this protocol by a regulatory agency is the responsibility of the Sponsor.

TEST PRINCIPLE

Inoculated carriers and appropriate chemical indicators (CIs), if applicable, are randomly placed within the sealed room. The inoculated carriers and CIs will be exposed to the test substance for a specified exposure time. After exposure, the carriers are transferred to vessels containing neutralizing subculture media and assayed for survivors. The CIs, if used, will be visually examined and observations will be recorded. Appropriate culture purity, viability, organic soil sterility, neutralizing subculture medium sterility, carrier population, HCl resistance, and neutralization confirmation controls will be performed.

TEST METHOD

Table 1:

Test Organism	ATCC #	Growth Medium	Incubation Parameters
Clostridium difficile - spore form	43598	CDC Anaerobic Blood Agar (or equivalent)	35-37°C, anaerobic

The test organism to be used in this study was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Preparation of Room Enclosure

The test room enclosure with dimensions 18'8.5" x 16'8" x 11'9", totaling approximately 3663.7 feet^3 or 104 m^3 will be used for testing. The room will be prepared by removing any dirt and waste substances visible to the naked eye, as applicable. This may involve sweeping and mopping the floor and washing down any visibly dirty surfaces. In addition, all porous surfaces including: fabrics, curtains, etc will be removed from the room. Shelves or other suitable means of supporting the test carriers and CIs will be positioned within the room.

The room enclosure will be sealed prior to testing in order to isolate the space in which the test substance will be applied. At a minimum this will include sealing the door exiting the room by any appropriate means. The HVAC vents in the ceiling of the test room may be covered, by Sponsor request. ATS Lab's standard HVAC system supplemented with a humidifier/dehumidifier may be used to reach the environmental conditions requested by the Sponsor prior to and during testing. In addition, supplemental fans may be used to assist with distribution of the test substance at the Sponsor's request.

Carriers

Brushed stainless steel disk carriers (diameter ≈1 cm, thickness ≈0.7 mm) will be used in this test. New stainless steel disk carriers will be soaked in a 1-5% solution of Triton X-100 for a minimum of one hour. The carriers will then be thoroughly rinsed at least 4 times in deionized water or until all soapy residue is gone. The carriers will then be soaked in 1.0 N NaOH for at least two hours to remove any residual soil. They will be rinsed until the rinse water is neutral to phenolphthalein then rinsed an additional two times in deionized water. All disks will be checked for pitting, rust or other defects prior to sterilization. All disks with defects will be discarded. Cleaned carriers will be placed into a sterile vessel then autoclave sterilized. Sterile stainless steel disks will be transferred to individual sterile Petri dishes matted with filter paper.

The minimum number of test carriers needed will be determined by the equation below.

Number of Test carriers = $[(m^3\text{-}10) / 2] + 15$

Where m^3 = the volume of the room enclosure in cubic meters

When using the test room with dimensions of 18'8.5" x 16'8" x 11'9", a minimum of 62 test carriers per organism will be used to substantiate disinfectant claims. An alternate number of carriers may be utilized at the Sponsor's request.

Chemical Indicators

Chemical indicators (CIs) in the form of test strips appropriate for the active ingredient may be supplied by the Sponsor. If they are used, they will be placed in the same locations as the test carriers. Color changes demonstrated by the CIs will be recorded following the completion of the test cycle. The use of CIs is strictly to verify the distribution of the test substance throughout the testing enclosure. They will not be used to provide a quantitative measure of test substance concentration.

Preparation of Test Organism

From a stock source (for example, a stock plate), inoculate sufficient 10 mL tubes of BHI broth with the test organism. Incubate the broth tubes for 2-5 days at 35-37°C under anaerobic conditions. Following incubation, vortex-mix the broth culture and inoculate sufficient CDC Anaerobic Blood agar (or equivalent) plates with 500 µL of broth culture per plate. Spread the inoculum over plates. Incubate the inoculated plates for 7-10 days at 35-37°C under anaerobic conditions. Following incubation, harvest the growth from the plates by adding 3 mL of sterile deionized water to each plate and gently scraping with a sterile cell scraper. Remove the liquid from each plate and transfer to a sterile vessel. Centrifuge-concentrate the suspension at 3400-3800 RPM for ≥ 20 minutes and concentrate the pellet in sterile deionized water. (An 8X concentration is recommended. Alternate concentrations may be used where appropriate.) Centrifuge-concentrate the suspension a second time at 3400-3800 RPM for ≥5 minutes. Remove the supernatant and resuspend the pellet in sterile deionized water (approximately equivalent to the volume of supernatant removed). Centrifuge concentrate the suspension at 3400-3800 RPM for ≥5 minutes a third time. Remove the supernatant and resuspend the pellet in sterile deionized water (approximately equivalent to the volume of supernatant removed). Macerate the culture to uniformity. The culture may be stored at 2-8°C for up to 6 months.

Prior to use in testing, heat an aliquot of working spore suspension for 10±1 minutes at approximately 65±2°C. Allow the suspension to cool to room temperature. The aliquot is then purified using a HistoDenz solution. Five (5.0) mL of 50% (w/v) HistoDenz in sterile deionized water is transferred to a sufficient number of 15 mL conical tubes. To each tube, approximately 1 mL of previously prepared spore suspension is pipetted to the top of HistoDenz solution. Centrifuge the tubes at approximately 2000 x g for approximately 20 minutes using a swinging bucket rotor. Following centrifugation, the top three layers (upper clear layer, dense second layer, and third clear layer) from each tube is removed by pipet and discarded. The fourth, mostly pelleted layer, is combined (as applicable) then resuspended by vortex mixing. One (1) mL volumes are transferred to microcentrifuge tubes. The microfuge tubes are centrifuged for approximately 5 minutes at approximately 16,000 x g. The supernatant is then discarded and the remaining pellet is resuspended in 1-1.5 mL of cold PBS +0.1% Tween 80 (ST80). The resuspended material is centrifuged for approximately 2 minutes at approximately 16,000 x g. The supernatant is again discarded and the remaining pellet is resuspended in 1-1.5 mL of cold PBS +0.1% Tween 80 (ST80). This step is repeated 2 more times for a total of three washes. Following the last wash, the supernatant is discarded and the pellet is resuspended in an appropriate volume of diluent to target approximately 2 x 10^8 spores/mL.

The spore suspension will be appropriately examined for quality by any suitable methods (examples: staining or phase contrast microscopy) and shall demonstrate a minimum 95% spore to vegetative cell ratio.

A standard organic soil load may be added to the test organism suspension at the Sponsor's request. Alternate soils may be used at the request of the Sponsor. If the standard soil load is requested, the soil load mixture will consist of tryptone, bovine serum albumin, and bovine mucin. For example, when preparing a 500 µL inoculum, add 25 µL of 5% bovine serum albumin and 100 µL 0.4% mucin and 35 µL 5% tryptone to 340 µL test organism suspension. Equivalent dilutions may be made.

Contamination of Carriers

The carriers will each be inoculated with 0.01 mL (10 µL) of a prepared suspension using a calibrated pipettor, uniformly spreading the suspension over the test surface. The dish will be covered immediately and the procedure repeated until all carriers have been inoculated. The contaminated carriers will be placed in a desiccator containing active desiccant. A vacuum will be drawn and the carriers will be dried for a minimum of 2 hours under ambient conditions. Carriers may be held in the desiccator containing active desiccant ≤30 days. The actual drying and/or storage time will be clearly documented.

Carrier Placement in the Room Enclosure

Inoculated and dried test carriers and CIs (if applicable) will be placed within the room to include at a minimum:

1. All corners of the room
2. Central locations on all wall faces
3. Central locations on the floor
4. Multiple locations and heights within the enclosed space.

Carriers will be placed in near vertical and horizontal positions throughout the room. CIs, if used, will be placed in a paired fashion with an inoculated test carrier. The approximate locations of test carriers, CIs (if applicable), vents, doors, application equipment, and fans (if used) will be documented. See protocol attachment for positioning diagram.

Test Substance and Equipment Preparation

The test substance(s) to be assayed will be used as directed by the Sponsor. If a dilution of the test substance is requested by the Sponsor, the test substance(s) will be diluted by ATS Labs and will be applied within three hours of dilution. The appropriate test substance volume will be added to the Sponsor provided application equipment and will be activated per Sponsor's instruction. Alternately, the Sponsor or Sponsor Representative may be present on the day of testing to prepare and activate the application equipment.

Exposure Conditions

Once each of the inoculated carriers is positioned and the required environmental conditions have been achieved, the lid of each Petri dish containing the inoculated carrier will be removed. The application unit will be activated, the technician will immediately exit the room, and the room will be sealed. The total time from activation to the completion of cycle will be documented and reported. The room exhaust will be verified to be off, unless otherwise directed by the Sponsor.

Following the completion of the test cycle, the room may be aerated as indicated by the Sponsor in order to assure the technicians may safely enter the room. If an aeration step is required, the total aeration time will be recorded and reported.

Test System Recovery

After exposure to the test substance, the treated test carriers will be individually transferred using sterile forceps to 10 mL aliquots of neutralizing subculture medium (representing the 10^0 dilution). The vessel containing the carrier will be sonicated for 5 minutes. Carriers may be scraped with a sterile device if inoculum is still visible on the surface after sonication. Following sonication, each carrier will be vortex mixed for approximately 45-60 seconds. The entire contents will be filtered by transferring the liquid from each vessel onto the surface of a filter membrane and evacuating the contents. Ten (10) mL of sterile saline will be added to the vessel containing the carrier and it will be vortex mixed. The rinse solution will be filtered using the same filter membrane as the 10^0 dilution. This rinse step will be repeated for a total of 3 rinses. Upon completion of the rinses, each filter membrane will be aseptically removed from the filter unit and placed onto the surface of an agar plate appropriate for the recovery of the test organism.

If CIs are used, they will be observed for color change and the results will be recorded.

Incubation and Observation

Subcultures will be incubated anaerobically at 35-37°C for 48±4 hours. If necessary, the subcultures may be stored at 2-8°C for up to three days prior to examination.

Upon completion of incubation the number of survivors will be enumerated. Representative subcultures showing growth may be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism.

STUDY CONTROLS

Purity Control

A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is growth of a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control

If applicable, the serum used for soil load will be cultured, incubated, and visually examined for lack of growth. The acceptance criterion for this study control is lack of growth.

Carrier Sterility Control
A representative uninoculated carrier will be added to the neutralizing subculture medium. A 1 mL aliquot of the suspension will be plated on appropriate agar, incubated, and visually examined for growth. The acceptance criterion for this study control is lack of growth.

Neutralizing Subculture Medium Sterility Control
A 1 mL aliquot of the uninoculated neutralizing subculture medium will be plated on appropriate agar, incubated, and visually examined for growth. The acceptance criterion for this study control is lack of growth.

Viability Control
A representative inoculated carrier will be added to the subculture medium. A 0.1 mL aliquot of the suspension will be plated on appropriate agar, incubated, and visually examined for growth. The acceptance criterion for this study control is growth.

Carrier Population Control
This control will be performed after the test exposure using inoculated carriers that were placed within a sealed container and held in the test room during the test substance application.

A minimum of three inoculated carriers for each inoculation/organism carrier set will be individually assayed. Each inoculated carrier will be subcultured into appropriate vessels containing 10 mL of neutralizing broth. The vessels will be sonicated and vortex mixed as in the test. Appropriate serial ten-fold dilutions will be prepared and duplicate 0.1 mL aliquots spread plated on agar plate medium, and incubated. Following incubation, the resulting colonies will be enumerated and the CFU per carrier calculated. The individual CFU per carrier results will be calculated, and the Log_{10} value of each carrier determined. The acceptance criterion for this control is a minimum geometric mean value of $\geq 1 \times 10^6$ CFU/carrier (≥ 6.0 Log_{10})

Neutralization Confirmation Control
The neutralization of the test substance will be confirmed by treating uninoculated carriers in the test room in parallel with the test. Upon completion of the treatment cycle, each carrier will be added to 10 mL of neutralizer (as in the test procedure). The contents will then be filtered and rinsed as in the test procedure into a filter unit. The filter will be inoculated with ≤500 CFU of test organism, evacuated, and plated. The same aliquot(s) of the test organism will be filter plated as a numbers control. This control may be performed using multiple dilutions of the test organism. The plates will be incubated with the test.

The acceptance criterion for this study control requires the neutralization control and corresponding population control results to be within ±1.0 Log. The control result will be reported using data from the most appropriate dilution.

HCl Resistance Control
Four inoculated and dried carriers will be added to individual vessels containing 10 mL of 2.5N HCl. After 2 minutes, 5 minutes, 10 minutes and 20 minutes, each carrier will be transferred to 10 mL of Modified Fluid Thioglycollate medium and mixed. A 0.1 mL aliquot will be plated from each neutralized tube and the plates will be incubated with the test. The acceptance criterion for this study control is growth after at least 2 minutes of exposure.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including test organism strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium, batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The U.S. EPA efficacy performance requirements for label claims require that the test substance demonstrate a minimum of a 6 Log_{10} or 99.9999% reduction in numbers of the test organism as compared to the carrier population control.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section. If any of the control acceptance criteria are not met, the test may be repeated under the current protocol number.

METHOD FOR CONTROL OF BIAS: N/A

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the organism strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES
If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study. Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

TEST SUBSTANCE RETENTION
It is the responsibility of the Sponsor to retain a sample of the test substance. All unused test substance will be discarded following study completion unless otherwise indicated by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation, and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. AOAC Official Methods of Analysis, Sporicidal Activity of Disinfectants, Eighteenth Edition, 2006, 966.04, Method I and II.
2. Association of Official Analytical Chemists (AOAC), 2005. Germicidal and Detergent Sanitizing Action of Disinfectants Method 960.09 [Preparation of Synthetic Hard Water].
3. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Efficacy Data Requirements, Disinfectants for Use on Hard Surfaces, DIS/TSS-1.
4. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1979. Efficacy Data Requirements, Supplemental Recommendations, DIS/TSS-2.
5. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, DIS/TSS-9, July 11, 1985
6. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Draft Protocol "Protocol for Sterilization of Porous and Non-Porous Surfaces within Sealed Enclosures using Vaporized Hydrogen Peroxide"
7. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, 1982. Subseries 91A Public Health Uses. *In* Pesticide Assessment Guidelines – Subdivision G (Product Performance).
8. U.S. Environmental Protection Agency, 2/2009. "Guidance for the Efficacy Evaluation of Products with Sporicidal Claims against *Clostridium difficile*".
9. American Society for Testing and Materials (ASTM). Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides, E 2197, 2005.
10. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2000: General Considerations for Public Health Uses of Antimicrobial Agents, March 12, 2012.
11. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2100: Sterilants - Efficacy Data Recommendations, March 12, 2012.
12. U.S. Environmental Protection Agency, Office of Chemical Safety and Pollution Prevention, Product Performance Test Guidelines, OCSPP 810.2200: Disinfectants for Use on Hard Surfaces- Efficacy Data Recommendations, March 12, 2012.

DATA ANALYSIS

Calculations

Number of Organisms Surviving per Carrier

$$\text{CFU/carrier} = \frac{\text{(average number colonies/plate @ dilution) x (dilution factor) x (volume neutralized solution)}}{\text{(number of carriers tested) x (volume filtered or plated)}}$$

The carrier population will be calculated and reported using data from the most appropriate dilution(s).

Geometric Mean of Number of Organisms Surviving on the Test or Control Carriers

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \ldots \text{Log}_{10}X_N}{N}$$

where X equals CFU/carrier and N equals the number of replicates tested

Percent Reduction = [(a - b) / a] x 100

Where: a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

Log Reduction = Log_{10} (a) – Log_{10} (b)

Where: a = geometric mean of the number of organisms surviving on the inoculated control carriers.
b = geometric mean of the number of organisms surviving on the test carriers.

$$\text{Spore Purity} = 100\% \times \frac{A}{A + B}$$

Where: A = mean spore count
B = mean vegetative count

Statistical Analysis:
None used.

STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Test Substance (Name & Batch Numbers, *including ≥60 day old batch* - exactly as it should appear on final report):
Sanosil S010 Lot# 12I001, 12060, 1109C

Specify ≥60 day old batch: 1109C **Expiration Date:** 3/21/12

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Peracetic acid
- ☒ Peroxide
- ☒ Other Silver

Test Substance Active Concentration (upon submission to ATS Labs): Hydrogen peroxide: ~4.75%, 85ppm Silver

Neutralization/Subculture Broth:
- ☑ Neutralizer: Letheen Broth + 0.07% Lecithin + 0.5% Tween 80 + 0.01% Catalase
  Recovery Agar: CCFA-HT Agar
- ☐ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:
- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other: _____

Hazards:
- ☐ None known: Use Standard Precautions
- ☑ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

Product Preparation
- ☑ No dilution required, Use as received (RTU)
- ☐ *Dilution(s) to be tested:

  _____ defined as _____ + _____
  (example: 1 oz/gallon) (amount of test substance) (amount of diluent)
  - ☐ Deionized Water (Filter or Autoclave Sterilized)
  - ☐ Tap Water (Filter or Autoclave Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____ PPM
  - ☐ Other _____

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism: ☑ *Clostridium difficile* - (spore form) (ATCC 43598)

Carrier Number: 62 per lot **Chemical Indicators Supplied:** ☑ Yes ☐ No

Name of Chemical Indicator: Sanosil H_2O_2 Strip Tests

Equipment Operational Instructions: Please refer to Halo User manual. ①
① If Sponsor representative is not present during testing MS 10-17-12

Cycle Time: ~140 minutes (estimated time from activation to 1 ppm H_2O_2 in the test room. Actual carrier subculture time will be reported)

Environmental Exposure Temperature Range: Ambient

Organic Soil Load:
- ☐ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☒ No Organic Soil Load Required
- ☐ Other: Example soil listed in protocol. (BSA, mucin, and tryptone)

TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in one or more previous studies at ATS Labs.
- ☐ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs: _____ Sent via *overnight* delivery? ☐ Yes ☐ No
- ☒ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs: 10/18/2012
- ☒ Sender (if other than Sponsor): CASE LABS, Whippany, NJ

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

- ☐ ① Approved without modification
- ☑ ② Approved with modification - Supplemental Information Form Attached - ☑ Yes ☐ No

② Updated per email to reflect carrier placement diagram attachment. MS 10-16-12

APPROVAL SIGNATURES

SPONSOR:

NAME: David Lach TITLE: Regulatory Scientist

SIGNATURE: _____ DATE: 10/15/2012

PHONE: 302-454-8102 FAX: 302-454-8009 EMAIL: dlach@sanosilglobal.com

For confidentiality purposes, study information will be released only to the Sponsor/Representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

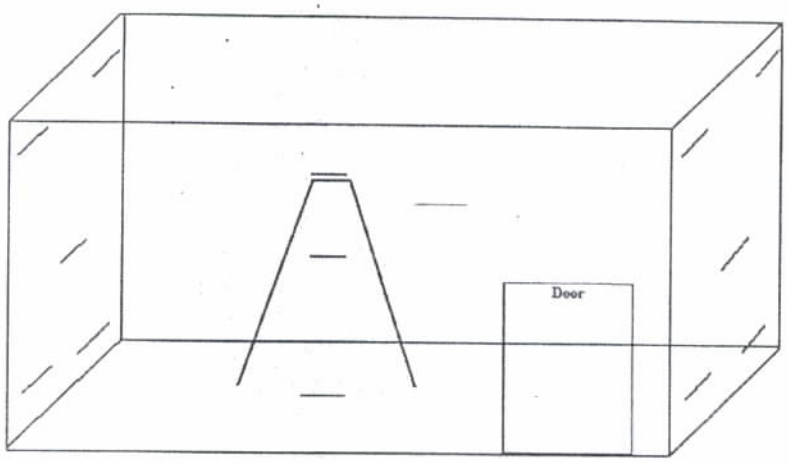
ATS Labs:

NAME: Matthew Sathe
Study Director

SIGNATURE: _____ DATE: 10-17-12
Study Director

Attachment To ATS Labs Protocol – SAN01101111.CUST

Carrier Placement Diagram



Carriers will be placed on the horizontal lines throughout the room. Horizontal lines near the floor represent horizontal carrier placement on the floor. Elevated lines in the upper corners and center wall faces will contain Petri dishes randomly placed in flat or near vertical positions. The application system and any needed accessories (humidifier, fans, etc.) are not included in the diagram at this time. They will be included in the location diagram included in the final report.